Cell Metabolism
Minireview

Bone Remodeling, Energy Metabolism, and the Molecular Clock

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The adult skeleton is constantly renewed through bone remodeling. Four recent papers (Baldock et al., 2007; Lee et al., 2007; Lundberg et al., 2007; Sato et al., 2007) provide new insights into central and peripheral control of this remodeling sequence. Two of the studies add to our knowledge of the complex hypothalamic modulation of bone turnover mediated by NMU and NPY via the sympathetic nervous system, while the other two focus on the peripheral neural target, the osteoblast, and its regulation by neuropeptides and osteocalcin. These findings support a new paradigm concerning the regulation of bone remodeling and provide a foundation for novel approaches to preventing osteoporosis.

Bone remodeling is a continuous process of skeletal renewal during which bone formation is tightly coupled to bone resorption. Skeletal turnover is essential for maintaining bone strength, regulating calcium homeostasis, and repairing microdamage incurred by normal weight-bearing activities. Bone remodeling also provides a framework for mobilizing calcium during exceptional physiological states. For example, calcium demands during lactation are intense, yet the skeleton can readily meet those needs by temporarily unbalancing the remodeling unit in favor of resorption. In contrast, cessation of lactation leads to a relatively rapid recovery of bone mass through enhanced bone formation and suppressed bone resorption.

Skeletal remodeling requires a significant amount of energy for the recruitment of osteoblasts and the deposition of collagen matrix. But precisely how bone turnover is coupled to energy metabolism was not clear until the turn of this century. In 2000, Ducy and colleagues (2000) made the seminal observation that hypogonadal *ob/ob* mice paradoxically exhibited very high bone mass. Subsequently, the Karsenty/Ducy laboratory demonstrated that leptin causes bone loss by activation of the sympathetic nervous system (SNS) rather than through circulating hormones or cytokines (Takeda et al., 2002). Identification of the β2-adrenergic receptor (β2-AR) as the target for sympathetic regulation of osteoblasts established a central regulatory loop (Takeda et al., 2002; Elefteriou et al., 2005). Fu et al. then identified a series of "clock" genes as prime osteoblastic targets for sympathetic activation by leptin (Fu et al., 2005; Karsenty, 2006). Within the past year, several new studies have further clarified the relationship between skeletal remodeling and the central nervous system (CNS).

In order to appreciate the significance of these recent findings, it is helpful to conceptualize the interaction between bone and brain as a regulatory unit composed of four phases (see Figure 1). For the skeleton to sense fuel status, afferent signals from fat storage depots are relayed to the hypothalamus (phase 1). In the ventromedial nucleus of the hypothalamus (VMH), complex neural processing occurs in conjunction with other neuronal circuits (phase 2). Glutamatergic, serotonergic, cannabinoid, and NPY-ergic pathways may also be activated by these afferent signals. In phase 3, efferent output through the SNS targets the β2 receptor on the osteoblast. The molecular pathways downstream of this activation include transcriptional, posttranslational, and chromatin regulation of clock genes that regulate osteoblast proliferation indirectly by inhibiting c-*myc*. This leads to suppression of cyclin D1, a critical cell-cycle protein. Alternatively, β2 receptor activation increases expression of the transcription factor AP-1 in osteoblasts, which stimulates c-*myc* activity, illustrating a putative "checks and balances" system for neural regulation of osteoblasts (Fu et al., 2005). Lee et al. (2007) recently demonstrated that osteoblasts can modulate adiponectin expression in adipocytes, completing the regulatory loop between bone and brain (phase 4). Four recent papers lend insight into three of the four phases: phase 2 (Baldock et al., 2007; Lundberg et al., 2007), phase 3 (Sato et al., 2007), and phase 4 (Lee et al., 2007).

In phase 1, fat cells control metabolic function by storing or releasing energy in response to nutrient intake. Adipocytes also synthesize cytokines, hormones, and growth factors that modulate the activity of other cells. Shortly after its cloning and identification, leptin emerged as a prime candidate for the energy sensor of adipocytes. It is a prototypic adipokine, exhibiting classic endocrine activity by crossing the blood-brain barrier, enhancing reproductive status, and reducing appetite via the arcuate nucleus (Spiegelman and Flier, 2001). Elegant studies have established that leptin regulates bone remodeling through the SNS by a relay in the VMH (Ducy et al., 2000; Takeda et al., 2002).

Adipocytes in the marrow can also secrete leptin, and therefore it is conceivable that leptin could influence adjacent stromal cells to enter the osteoblast lineage. However, the importance of this direct effect has been called into question because targeted overexpression of leptin in bone does not produce a skeletal phenotype (Astudillo et al., 2007; Takeda et al., 2002). Other adipokines, such as adiponectin, promote insulin sensitivity and enhance osteoblastic proliferation but do not regulate sympathetic activity (Berner et al., 2004). Fat cells synthesize other neuropeptides, such as PYY, that bind to peripheral Y receptors in osteoblasts. But leptin remains the primary energy sensor in fat tissue, regulating hypothalamic efferent output to the skeleton in a manner similar to its effects on appetite centers in the brain.

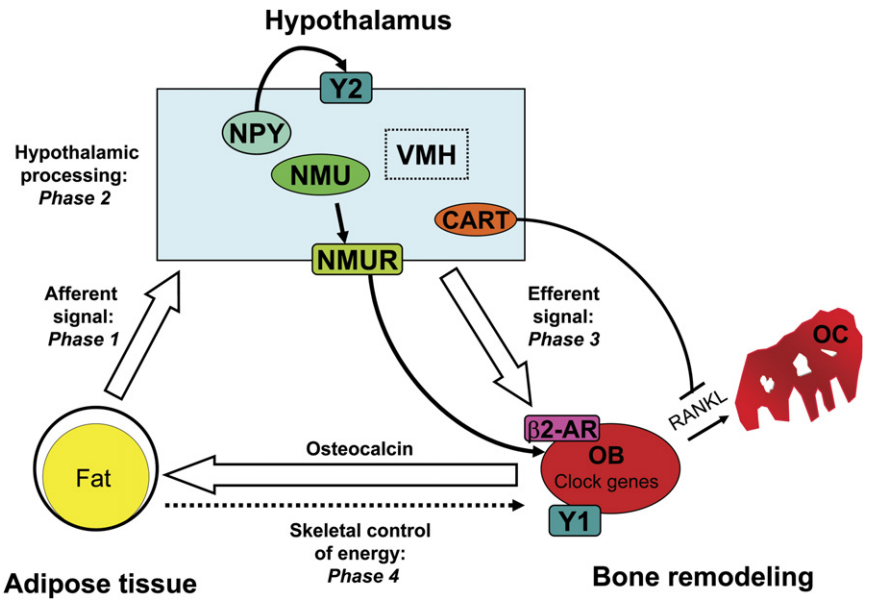

Figure 1. Central Regulation of Bone Remodeling

Central regulation of bone remodeling occurs through the hypothalamus but is determined by both afferent and efferent signaling. Phase 1 is the afferent leptin signal that originates from peripheral adipocytes. Phase 2 involves the processing of this signal in the hypothalamus, which occurs in the ventromedial hypothalamus (VMH). The mediators of this phase likely include neuropeptide Y (NPY) and neuromedin U (NMU). Phase 3 represents the efferent (sympathetic) output from the hypothalamus to the β2-adrenergic receptor (β2-AR) on osteoblasts and the resultant change in transcription factors and clock genes that affect osteoblastogenesis. Suppression of osteoclastogenesis can occur indirectly through the suppression of receptor activator of NF-κB ligand (RANKL) in osteoblasts by cocaine- and amphetamine-regulated transcript (CART). NMU may mediate its effects downstream of β2-AR on the clock genes. Phase 4 represents skeletal regulation of adipocytes, most likely through the systemic release of osteocalcin. The dotted line in phase 4 represents the theoretical possibility that adipocytes could regulate osteoblast proliferation and differentiation. Other abbreviations: Y1, NPY receptor 1; Y2, NPY receptor 2; NMUR, neuromedin U receptor 2; OB, osteoblast; OC, osteoclast.

Central control of appetite, reproduction, and bone remodeling (phase 2) is a complex process, but progress in understanding it has been evident. Previously, it had been shown that chemical sectioning of the VMH with gold thioglucose replicates the high bone mass and bone formation phenotypes of the *ob/ob* mouse (Karsenty, 2006). Similarly, intracerebroventricular (i.c.v.) leptin infusions to *ob/ob* mice with their arcuate nuclei sectioned by monosodium glutamate corrected the bone mass but did not change body weight, suggesting distinct sites for regulation of remodeling and appetite (Takeda et al., 2002).

Melanocortin, part of a family of peptides produced by post-translational processing of pro-opiomelanocortin (POMC), had originally been considered to be the major signaling molecule mediating bone turnover. Two receptors for melanocortin are present in the arcuate nucleus, and gene deletion of one leads to mice with high bone mass and suppressed bone resorption (Elefteriou et al., 2005). Mice with the melanocortin receptor (MC4R) deletion show increased levels of another neuropeptide, CART (cocaine- and amphetamine-regulated transcript), which is also expressed in the arcuate nucleus and is regulated by leptin (Ahn et al., 2006). Interestingly, CART suppresses expression of receptor activator of NF-κB ligand (RANKL), which is produced by osteoblasts and is essential for osteoclast differentiation (Ahn et al., 2006). In the *ob/ob* mouse, bone formation is increased, as is bone resorption, and CART expression in these mice is absent in the hypothalamus. However, CART-deficient macrophages and stromal cells differentiate normally, and there are neither CART receptors nor evidence for CART expression in bone, suggesting the presence of other central mediators of bone turnover (Ducy et al., 2000; Elefteriou et al., 2005).

Neuropeptide Y (NPY) has been the focus of recent work by the Herzog group. NPY belongs to a class of peptides including PP and PYY that are expressed in the central and peripheral nervous systems as well as in the gut. They signal through five distinct receptors (Y1, Y2, Y4, Y5, and Y6) expressed in various tissues. Like melanocortin and CART, NPY is synthesized in the arcuate nucleus and increases appetite when overexpressed. NPY-immunoreactive fibers have been identified within bone marrow and the periosteum and preferentially around vascular elements that surround marrow cells. Moreover, NPY treatment of wild-type osteoblasts in vitro reduces cell proliferation. Previously, it was shown that mice with either a germline or hypothalamic deletion of the Y2 receptor had increased cancellous and cortical bone mass as well as greater bone formation, albeit no change in bone resorption. Recently, Baldock et al. (2007) performed germline deletion of Y1 receptors in mice and found that these mice had high bone mass; however, when they performed targeted deletion of Y1 genes only in the hypothalamus, bone density was not altered. In an accompanying paper published by the same group, marrow stromal cell proliferation was greatly enhanced in the germline $Y2^{-/-}$ mice, and this was accompanied by in vitro evidence of increased mineralization (Lundberg et al., 2007). Surprisingly, these mice had virtually no Y1 receptors in bone cells. Taken together, data from these two studies suggest that central NPY signaling may modulate bone remodeling but that the anabolic effect of the *Y2* gene deletion is mediated by peripheral regulation of the Y1 receptor on osteoblasts. Whether this occurs through NPY-ergic pathways emanating from the hypothalamus or through other central pathways has yet to be determined.

One interesting sidelight to the *Y2* null studies by Lundberg et al. (2007) was the observation that marrow stromal cells from the mutants more readily differentiated into adipocytes under the appropriate culture conditions than wild-type cells did, even though the mutant mice had a lean phenotype with reduced white adipose tissue and relatively fewer marrow adipocytes than controls. The most likely explanation for this finding is that in vitro studies do not reflect in vivo circumstances. However, these data might be interpreted as indicating that marrow stromal cells from $Y2^{-/-}$ mice are more pluripotent than wild-type

stromal cells. Indeed, if there is bidirectional communication between adipocytes and osteoblasts, it is conceivable that some stromal cells could become adipocytes in order to provide a source of energy for sustaining stromal cell differentiation into osteoblasts. An example of the coexistence of marrow adipogenesis and osteoblastogenesis is found in *Ebf1* (early B cell factor 1) null mice. These mice, on a mixed genetic background, have markedly increased bone formation and high bone mass, even though their bone marrow has an overabundance of adipocytes (Horowitz and Lorenzo, 2007).

Phase 3 represents the efferent output of sympathetic activity from hypothalamic signals to the osteoblast. How is this accomplished? Another recent study (Sato et al., 2007) provides insight into this critical question. Neuromedin U (NMU) is a neuropeptide produced by nerve cells in the brain, including the dorsomedial nucleus of the hypothalamus and the pituitary, and in the submucosal and myenteric plexuses of the small intestine. It binds to NMU receptor 2 (NMUR2) in the hypothalamus and, like other neuropeptides, can regulate appetite control and sympathetic activation. In *ob*/*ob* mice, NMU is deficient but is corrected by leptin treatment. Sato et al. studied $NMU^{-/-}$ mice and found high bone mass with increased bone formation, although isolated osteoblasts and osteoclasts proliferated and differentiated normally. These data implied that NMU must act centrally to control remodeling. However, i.c.v. leptin infusion or β-adrenergic agonist administration to $NMU^{-/-}$ mice did not reduce bone mass, suggesting that NMU may actually be downstream of leptin and the SNS. Indeed, Sato et al. also reported that central leptin administration paradoxically increases osteoblast numbers in $NMU^{-/-}$ mice. These findings, along with the discovery that $NMU^{-/-}$ mice have high levels of the urinary catecholamine metanephrine but increased bone mass and normal bone resorption, imply either that the absence of NMU leads to skeletal resistance to sympathetic activation by leptin or that a compensatory change occurs in other neuropeptides, thereby balancing any increase in sympathetic tone. Interestingly, CART expression was increased in the $NMU^{-/-}$ mice, implying that there must be a fine balance between the suppressive effects of CART on osteoclastogenesis and the bone-resorbing activity induced by sympathetic activation.

Why was bone formation enhanced in these mice if $NMU^{-/-}$ osteoblasts and osteoclasts proliferate and differentiate normally in vitro? Sato et al. found one clue in the clock genes *Per1* and *Per2*, whose expression was significantly downregulated in the *NMU* null mice. This finding was reminiscent of osteoblasts from the *ob*/*ob* mice, in which absence of β2-AR signaling due to leptin deficiency causes reduced clock gene expression and a subsequent increase in bone formation. However, there are limitations when comparing in vitro data with in vivo data. Therefore, the exact mechanism causing the high bone-mass phenotype in the $NMU^{-/-}$ mice remains to be determined.

A key to understanding central modulation of peripheral remodeling are the clock genes, which encode a family of proteins expressed in a circadian pattern. Type I collagen and osteocalcin exhibit significant periodicity during a 24-hour cycle, as do osteoprotegerin (OPG, an endogenous inhibitor of RANKL) and parathyroid hormone (Gundberg et al., 1985; Joseph et al., 2007). Interestingly, adipokines, including leptin, adiponectin, and resistin, also show circadian rhythmicity (Kalsbeek et al., 2007). The master clock generator is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. It entrains environmental cues, such as light (via retinal neurons), feeding activity, and hormonal signals, to regulate peripheral clock genes through neuronal signals. At the molecular level, the clock engine is characterized by two transcription factors, Bmal1 and Clock. These proteins heterodimerize to regulate the expression of several other circadian genes such as the periods (*Per1* and *2*), the cryptochromes (*Cry1* and *2*), *Rev-erbα*, *Rorα*, and *nocturnin.* To complete this regulatory loop, Per and Cry form a complex that negatively feeds back on *Bmal1* and *Clock*. Gene deletion of *Per 1* and *2* or *Cry 1* and *2* results in mice with a high bone-mass phenotype but without major changes in body composition or other overt metabolic disturbances (Fu et al., 2005). Additionally, these null mice have normal serum leptin levels, and sympathetic activation is intact. This implies that the molecular clock for regulating osteoblast-specific genes is downstream of β2-AR and is an important mediator of phase 3 in the central control pathway (Figure 1). Interestingly, in contrast to deletion of other clock genes, mice with global knockout of *nocturnin* have a body composition phenotype that includes leanness with enhanced insulin sensitivity and reduced periodicity of the *Pparg* gene in response to high-fat feeding (Green et al., 2007). Skeletal phenotyping of these mice should provide further insight into the downstream effects of this clock gene on bone mass and body composition.

In phase 4, osteoblasts regulate adipocyte function through secretion of one or more mediators. If adipocytes can sense energy and relay this to a central control network for fine tuning bone remodeling, can the skeleton regulate fuel status in a systemic manner? Lee and colleagues (2007) have now convincingly demonstrated that a soluble factor produced from fully differentiated osteoblasts, but not fibroblasts, enhances insulin signaling when islet cells are cocultured with bone cells. Just as importantly, if adipocytes are cocultured with mature osteoblasts, adiponectin expression in the adipocytes is also markedly increased. Based on earlier mouse and human studies, Lee et al. hypothesized that osteocalcin was the soluble mediator responsible for influencing adipocytes and islet cells. To test this hypothesis, they crossed mice with one allele of the osteocalcin gene deleted with mice that were deficient in *Esp* (*Ptprv*), a protein tyrosine phosphatase expressed in Sertoli cells and osteoblasts. $Esp^{-/-}$ mice have profound hypoglycemia and increased insulin sensitivity, but as a result of removal of one copy of the osteocalcin gene, the hypoglycemic phenotype is ameliorated. Furthermore, Lee et al. demonstrated that the uncarboxylated form of osteocalcin is the most likely systemic stimulator of insulin secretion. The implications of this landmark paper cannot be overstated: for the first time, there is evidence that the skeleton can actively regulate energy metabolism. Clearly, more work needs to be done to define the target receptor on islet cells and adipocytes for osteocalcin and to determine the significance of the uncarboxylated form of this peptide "hormone." But functional linkage between bone and fat has been established.

Although there is now incontrovertible evidence for the neural control of bone remodeling, it is worthwhile to consider the implications of these new findings. There are a number of clinical

scenarios in which central neural pathways are disordered, leading to altered energy utilization and changes in bone mass. For example, patients with Cushing's disease or individuals with severe depression have high serum cortisol levels, reduced bone formation, fat redistribution, and a disruption in circadian rhythms. Interestingly, a similar phenotype is seen in individuals with chronic opioid addiction and patients with schizoaffective disorders treated with newer agents such as olanzapine. If these conditions represent a resetting in hypothalamic control of bone remodeling, how are the efferent sympathetic signals to the skeleton sensed, and do these changes affect the clock genes of the osteoblast? Also, are other neural pathways, such as those in the dopaminergic and serotonergic systems, modulating bone turnover in these conditions? There is preliminary evidence suggesting that serotonin is important in the central control of remodeling and that gene deletion of the major serotonin receptor in osteoblasts, *5HT2BR*, results in low bone mass and impaired osteoblast proliferation (Collet et al., 2007). In the same light, osteoblasts express both cannabinoid (CB1 and 2) and opiod (mu) receptors. Agonists of CB2 have been shown to increase bone mass, while deletion of the central *CB1* receptor gene leads to high bone mass, probably as a result of impaired sympathetic activation (Ofek et al., 2006). The recurring theme, that bone cells express receptors for numerous neuropeptides, reinforces the thesis that the CNS is a critical modulator of bone turnover.

These studies also raise new questions about the function of marrow adipocytes relative to neighboring osteoblasts. Marrow fat in adults can be measured by MRI and is now considered a risk factor for low bone mass and fractures (Shen et al., 2007). Age-related marrow adiposity may be due to a compensatory or default mechanism whereby marrow fat fills space previously occupied by bone, presumably because mesenchymal stem cells enter the adipocyte lineage rather than the osteoblast differentiation scheme. But it is also conceivable that marrow adipogenesis may be dynamic, adapting to energy needs and shifting its function depending on a particular environmental condition in the marrow niche. For example, in anorexia nervosa, despite the absence of peripheral fat, the marrow has an overabundance of adipocytes (Abella et al., 2002). Is this a response to impaired bone formation from a central mechanism, or is this an evolutionary response to protect the skeleton and maintain function in the absence of a systemic energy source?

In summary, recent findings have moved central control of bone remodeling and its relationship to energy status into the forefront of skeletal and metabolic research. We can expect even more provocative findings in the future, as new therapies such as NMU antagonists are considered for targeting the central regulatory circuits involved in bone remodeling.

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